SWEDISH CONIFEROUS FOREST PROJECT

BARRSKOGSLANDSKAPETS EKOLOGI

TECHNICAL REPORT 12 1977

MONTHLY ESTIMATIONS OF SOIL BACTERIA AT JÄDRAÅS AND A COMPARISON BETWEEN A YOUNG AND A MATURE PINE FOREST ON A SANDY SOIL

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MONTHLY ESTIMATIONS OF SOIL BACTERIA AT JÄDRAAS AND A COMPARISON BETWEEN A YOUNG AND A MATURE PINE FOREST ON A SANDY SOIL

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Abstract

Bacterial numbers and biomass estimated by direct counting of monthly samples in a mature pine forest on a sandy soil (Ih V) showed a large peak in the fall and a smaller one in the spring. The autumn peak coincided with the main pine needle fall. The mean bacterial biomass down to 10 cm from the litter surface was 17.5 g dw m^{-2} of which an average of 42% was found in the litter and humus layers. A sampling down to 50 cm depth showed that a substantial amount of the biomass was found deeper down in the profile. Only 14% of the biomass was found in the two organic layers where 50% of the oxygen was consumed, indicating a higher metabolic activity in the upper part of the profile.

1. Introduction

This work reports the bacterial part of the monthly sampling programme made from May 1974 to July 1975 at Jädraås, the main site of the Swedish Coniferous Forest Project. Samplings of the main components of the soil fauna were made on the same occasions (arthropods - T. Persson, enchytraeids - H. Lundkvist, nematodes - B. Sohlenius) and every three months the fungal hyphal length and biomass were estimated (E. Bååth and B. Söderström). A comparison between Ih VA (125-year-old pine forest) and Ih II (a 15-year-old pine forest) was also made as well as a sampling every 10 cm down to 50 cm at Ih VA.

2. Materials and methods

Samples were taken from squares (40:40), (90:40), (50:70), (10-120), (10:170) and (60:170) at Ih VA on the dates indicated in Table 1. A corer with a diameter of 67 mm was used and each time one core from

each of the six squares was sampled. The intact cores were wrapped in plastic and brought back to the laboratory where they were processed within 3-4 hours of sampling. The cores were separated into litter layer (A_{00}), humus layer ($A_{01} + A_{02}$), bleached layer (A_2) and the enriched mineral layer (B, 1-4 cm from the boundary to the bleached layer). Corresponding layers from the six cores were then pooled. After handmixing, 2.5 g of the organic and 10 g of the mineral layers were used for the determinations, which were made by direct counting (x 1000) in UV light after staining with acridine orange. The biomass was calculated after size measurements of 30 cells per sample assuming the same size distribution throughout the sample using a density of 1 and a dry weight equal to 20% of the fresh weight. To calculate the figures per square metre, the weights of the layers according to Staaf (pers. comm.) were used (A_{00} 707 g, $A_{01} + A_{02}$ 1990 g; mineral layers: 1-10 cm 99 400 g, 10-20 cm 120 600 g, 20-30 cm 133 000 m^{-2} g). The respiration was measured in a Gilson respirometer and the plate counts were made on Tryptone Soy Agar (Oxoid) plates.

On each sampling occasion the moisture content was determined on separate samples by drying to constant weight at 105°C. Loss on ignition was determined after heating to 550°C for 24 h. Precipitation values were obtained from the standard meteorological recordings in the project.

3. Results

Both bacterial numbers (Fig. 1) and biomass (Fig. 2) showed a small peak in the early spring and a much larger one in the early fall. The highest values were noted in September. They were approximately seven times higher than the minimum in June. By October the values had decreased to half of the maximum of the previous month. Throughout the winter the populations remained at fairly constant levels of about twice the minimum value. With rising soil temperatures a biomass peak was noted in April followed by a decrease in May together with decreasing moisture content. The values for all layers increased and decreased simultaneously. The mean values m^{-2} over the fourteen months were 1.2 g dw in the litter layer, 6.1 g in the humus layer, 3.3 g in the bleached layer and 6.9 g down to 10 cm from the surface of the mineral soil, which gives a sum of 17.5 g per m^2. The per cent distribution of the biomass between the organic and mineral layers down to 10 cm is shown in Table 1. For the

organic layer the lowest fraction found was 14%, in July 1974. The maximum was reached in October the same year with 60%; the mean was 42%. The amount of biomass per cm depth (Table 1) was more or less constant with the exception of September and October. In September increased bacterial amounts were recorded in the mineral layer and in October an increase was noted for the organic layers.

Plate counts were made on the same samples from October onwards (Table 2). For the organic layers the values for October were ten times higher than the means for the whole sampling period. The humus layer showed a higher mean than the litter layer in agreement with the data obtained by direct countings and the bleached layer contained more bacteria than further down in the mineral soil.

Table 3 shows data from a comparison between Ih VA and Ih II. The young stand (Ih II) had a lower oxygen consumption rate in the organic layers whereas in the mineral layers the values for the two sites were alike. The vertical distibutions of bacterial numbers, biomass, oxygen consumption rate and organic matter down to 50 cm at Ih VA are also given in Table 3. When calculated on an areal basis, 33% of the biomass was found in the top ten centimetres but it consumed 70% of the oxygen (Table 4). Fig. 3 shows the oxygen consumption rate of samples of the humus layer adjusted to different moisture contents at $15^{o}C$ and at $20^{o}C$. Calculation of respiratory Q_{10} values from Table 3 will give values between 3 and 4 in the interval $5^{o}C$ to $15^{o}C$.

4. Discussion

A small increase in bacterial numbers and biomass in the spring and a larger peak in the fall is generally found with monthly sampling (*e.g.* Gray *et al.*, 1973). Short term fluctuations in the bacterial populations can be quite large and rapid, however, when the environmental conditions are changed (Clarholm, 1977). The size of the change is the result of the combined influence of moisture, temperature and the availability of inorganic nutrients and carbon sources. Increases in temperatures are probably most important at the beginning and end of the growing season when the moisture is nonlimiting (Fig. 2) and temperatures low. During the summer months the moisture usually limits the rate of biological activity. Both Fig. 3 and estimations of bacterial numbers and biomass

at different natural moisture contents (Clarholm, 1977) show that water becomes a nonlimiting factor at around 200% moisture content. Such high values are rare during the summer. After a rainfall (2-3 mm) an increase in bacterial biomass has been noted with a three to four days delay; the peak is followed by a fairly rapid decline which could be caused by an actively grazing soil fauna. Thus the monthly values can be regarded as representative of the influence of the 3-4 previous days. In August 1974 a daily sampling series was carried out (Clarholm, 1977). Within fourteen days the bacterial numbers fluctuated three-fold and the biomass four-fold and changes were positively correlated to the changes in moisture. Table 1 shows the total precipitation on the four days preceding each sampling. In August and September the rainfall on these four days was sufficient to increase the moisture almost to a nonlimiting level and far above average for the summer months, thus contributing to the high values found in those months. Another reason for the high bacterial values is the carbon input from the fall of pine needles. Of the total yearly needle fall, 70% takes place in the period from late August to early November with some variation year from year depending on weather conditions. In 1974, the needles started to fall during the last week of August with a peak during the two first weeks of September. The period of needle fall ended with a storm in the first week of October. The mean needle fall per day was 1.3 g with maximum values of 1.8 to 2.2 g in September (all information about needles from J. Flower-Ellis, pers. comm.). Considering that the whole input of organic matter to the humus layer per year and m^2 is 374 g (branches included) (H. Staaf, pers. comm.) this is a large input in a short time when the temperature is still high and the moisture within favourable limits. As the needles still have nearly their normal content of carbohydrates when they fall, sugars and other easily metabolized carbon rich substances, which are quickly leached, they can serve as a substantial carbon source for the energy limited bacterial populations. The fungal biomass of the humus layer, which was measured by the Jones and Mollison technique in August 1974 was twice as large as in both May and November (B. Söderström, pers. comm.).

The content of bacteria per centimetre depth is surprisingly uniform in the top ten centimetres (Table 1), but since more than 50% of the oxygen consumption took place in the organic layers the bacteria there are possibly more active. The higher consumption rate might also be due

to the activities of other groups of organisms, *e.g.* fungi. If the values of oxygen consumption are converted into the amount of carbon evolved m^{-2} h^{-1} we get a value of 0.4 g, which is far higher than the 0.03 g reported for the total soil respiration by Staaf (1976). It has to be remembered that the samples used in the Gilson respirometer were mixed and that the observation is from a single day in comparison with the mean of a series of measurements of total soil respiration. The two methods agree fairly well, however, on the proportions of the respiration taking place in the organic and mineral layers, respectively.

Bacterial numbers, biomass, oxygen consumption and moisture content of samples from Ih VA on two consecutive days (July, 22-23) are given in Table 3. The first days samples with a small biomass had a large oxygen consumption and the second day samples, with an almost twice as large biomass, respired much less in spite of more favourable moisture conditions than the day before. This might be explained by the earlier observation (Clarholm, 1977) that there is a peak in bacterial biomass 3-4 days after a rainfall. 4.5 mm of rain fell on July 19-20, which would lead to a peak around the 23rd. The high oxygen consumption observed on May 22 represents a rapidly growing bacterial community. This suggestion is highly speculative and assumes that bacteria make up by far the largest component of the quick response to increased moisture.

The differences between Ih VA and Ih II (Table 3) could be explained by the differences in moisture contents of the organic layers. The moisture in the A_{00} layer at Ih II was only one-fourth of that in Ih VA and the figure for the humus was 50% lower. Oxygen consumption values for the old stand at similarly low moisture contents registered at other occasions are of the same magnitude (Fig. 3). The moisture differences can be explained by the less dense field layer and the poorly developed moss carpet at Ih II. The lower moisture content is also enough to explain the differences found in bacterial numbers and biomass. Measurements of metabolicly active amounts of fungal hyphae (fluorescein diacetate technique) showed no significant differences (B. Söderström, pers. comm.).

5. References

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STAAF, H. 1976. Koldioxidavgivningen från marken i ett 120-årigt tallbestånd på Ivantjärnsheden, Gästrikland - preliminära resultat från sommaren och hösten 1974. Swedish Coniferous Forest Project. Internal Report No 36.

Table 1. Bacterial biomass in g dw and numbers of bacteria x 10^{-13} (in parenthesis) per m^2 cm^{-1} plus moisture in the sample in per cent of the dry weight in four layers of Ih VA over a fifteen months period 1974-1975. The precipitation the four preceding days before the sampling date and the percentage distribution of biomass between the organic and inorganic layers are also given

Sampling date	Precipitation (mm)	Litter layer A_{00}	Humus layer $A_{01}+A_{02}$	Bleached layer A_2	Mineral layer B	% biomass in the organic layers ($A_{00}+A_{01}+A_{02}$)
May 21 1974	0	0.6 (0.7) 51%	0.6 (0.4) 41%	0.7 (0.7) 7%	0.7 (0.9) 11%	43
June 25	13	0.2 (0.2) 40%	0.3 (0.3) 52%	1.1 (0.8) 8%	0.4 (0.6) 7%	27
July 22	0	1.0 (0.6) 48%	0.7 (0.5) 111%	2.4 (1.3) 12%	2.1 (1.2) 12%	14
Aug 20	4.1	2.6 (1.4) 56%	4.3 (3.1) 171%	2.4 (2.0) 10%	2.1 (3.1) 10%	40
Sept 20	2.7	3.5 (2.5) 167%	3.0 (1.5) 184%	5.2 (4.4) 16%	6.2 (4.4) 10%	29
Oct 17	7.0	2.9 (2.8) 306%	2.6 (2.5) 275%	0.8 (1.0) 16%	1.6 (1.5) 16%	59
Nov 20	14.8	1.4 (1.5) 177%	1.7 (1.5) 188%	0.9 (1.0) 16%	1.3 (0.9) 14%	42
Dec 16	6.0	0.7 (0.8) 327%	1.2 (1.0) 248%	1.0 (1.3) 16%	0.8 (1.0) 14%	46
Jan 21 1975	27.6	0.7 (0.5) 338%	1.2 (1.0) >500%	1.0 (1.0) 22%	1.0 (0.8) 19%	48
Feb 20	2.2	0.5 (0.6) 232%	1.5 (1.2) >500%	1.1 (1.2) 18%	0.9 (0.9) 11%	48
April 29	0.9	1.9 (1.5) 265%	2.7 (2.1) >500%	1.4 (0.9) >30%	1.6 (1.1) >30%	56
May 27	7.7	0.9 (0.9) 185%	1.7 (1.7) 219%	0.9 (1.2) 14%	1.1 (1.3) 15%	50
June 25	1.1	0.5 (0.6) 32%	1.0 (1.2) 80%	1.0 (1.4) 7%	1.0 (1.6) 9%	58
July 23	4.5	1.4 (1.2) 131%	2.1 (1.9) 151%	1.2 (1.3) 7%	0.7 (0.8) 8%	63
Mean values		1.3 (1.1)	1.8 (1.4)	1.5 (1.6)	1.5 (1.4)	42

Table 2. Numbers of bacteria per g dw at Ih V estimated by plate counts at the monthly samplings, October 1974 - July 1975.

	A_{00} 10^{-6}	$A_{01}+A_{02}$ 10^{-6}	A_2 10^{-6}	B 10^{-6}
Oct 17	201	217	0.45	0.17
Nov 20	9	10	1.18	0.38
Dec 16	35	41	2.99	1.12
Jan 21	6	92	0.71	0.85
Feb 20	45	24	0.28	0.36
April 29	19	22	1.80	0.63
May 27	31	10	1.11	1.62
June 25	4	3	0.40	0.35
July 22	17	16	1.10	0.83
July 23	24	33	1.10	0.87
Mean	39	47	1.04	0.71
July 23 Ih II	15	30	7.70	15.1

Table 3. Comparison of bacterial numbers (direct and plate count), respiration and soil characteristics of different depths in the soil profile at Ih VA (samples from four sample points were pooled) and between Ih VA and Ih II (six pooled samples).

Site and horizon sampling date	Respiration $l O_2 g^{-1} h^{-1}$ 5°C	10°C	15°C	Soil moisture % of dry weight	pH_{H_2O}	Soil organic matter (% loss of ignition)	Bacterial numbers g^{-1} dw x 10^{-10} direct count	Bacterial biomass mg dw g^{-1} dw direct count	Bacterial numbers plate count no g^{-1} x 10^{-6}
Ih VA May 22 1975									
A_{00}-layer			95	98		96	1.67	1.8	17
$A_{01} + A_{02}$-layer			155	136		87	1.99	2.2	16
Mineral soil 0 cm			5.1	11		44	0.14	0.15	1.1
10 cm			1.0	7		4.1	0.14	0.12	1.8
20 cm			0.61	5		2.4	0.08	0.06	0.8
30 cm			0.33	3		1.3	0.08	0.05	0.7
40 cm			0.26	2		0.9	0.07	0.04	1.0
50 cm			0.18	2		0.8	0.02	0.01	0.7
Ih VA July 23 1975									
A_{00}-layer	30	58	87	131	4.5	99	1.74	2.0	24
$A_{01} + A_{02}$-layer	26	52	90	151	4.3	90	3.32	3.7	33
A_2-layer	0.5	1.0	1.9	7	4.5	3.9	0.13	0.12	1.1
B-layer	0.3	0.8	1.3	8	4.5	4.4	0.08	0.07	0.9
Ih II July 23 1975									
A_{00}-layer	1.6	5.1	9.5	35	4.6	90	1.01	1.2	15
$A_{01} + A_{02}$-layer	10	21	38	98	4.1	77	1.44	1.0	30
A_2	0.4	1.1	1.7	13	4.8	2.9	0.14	0.10	0.8
B	0.3	0.7	1.0	13	4.9	40	0.16	0.10	1.5

Table 4. Distribution of bacterial biomass, organic matter and respiration (measured by Gilson respirometry) per square metre at Ih VA. Four pooled samples, sampling date 1975-07-22 (compare Table 3).

Layer	Depth from surface (cm)	Bacterial biomass g	% of total	ml O_2 h^{-1}	% of total	amount organic matter g	organic matter content g cm^{-1}
A_{00}	0-1	1.3	3.2	67.2	8.9	703	703
A_{01}	1-4.5	4.4	10.9	309.2	40.8	1537	439
A_2	4.5-6.5	3.0	7.4	101.4	13.4	508	254
B	6.5-8.5	2.8	6.9	39.6	5.2	508	254
	8.5-10	1.9	4.8	14.9	2.0	381	254
	10-20	10.9	27.0	96.5	12.7	2145	215
	20-30	6.7	16.5	59.9	7.9	1013	101
	30-40	6.0	14.9	39.9	5.3	n.d.	
	40-50	3.3	8.3	29.3	3.9	n.d.	
Total		40.2	100	757.8	100		

Table 5. Monthly estimates of bacterial numbers and biomass per g dw in the A_{00}, $A_{01}+A_{02}$, A_2 and B layers of Ih VA. For construction of $A_{01}+A_{02}$ (mean) see Table 6.

Horizon / Month	A_{00}		A_{01}		$A_{01}+A_{02}$ mean		A_{02}		A_2		B	
	no per g dw x10^{-10}	biomass mg dw per g dw	no per g dw x10^{-10}	biomass mg dw per g dw	no per g dw x10^{-10}	biomass mg dw per g dw	no per g dw x10^{-10}	biomass mg dw per g dw	no per g dw x10^{-10}	biomass mg dw per g dw	no per g dw x10^{-10}	biomass mg dw per g dw
May 1974	0.95	0.91			0.76	0.94			0.07	0.07	0.09	0.07
June	0.26	0.33							0.08	0.11	0.06	0.04
July	0.85	1.36			0.82	1.20			0.13	0.29	0.12	0.21
Aug	1.92	3.61			5.48	7.52			0.20	0.43	0.31	0.48
Sept	3.56	4.88			2.69	5.23			0.44	0.52	0.44	0.62
Oct	3.92	4.14			4.31	4.59			0.10	0.08	0.15	0.16
Nov	2.16	1.97	2.96	2.97	2.72	2.93	1.99	2.79	0.10	0.09	0.09	0.13
Dec	1.17	0.95			1.92	2.04			0.13	0.10	0.10	0.08
Jan	0.66	0.97			1.78	2.17			0.10	0.10	0.16	0.13
Feb	0.87	0.78	2.34			.70	1.66	1.58	0.12	0.11	0.09	0.09
Mars	-	-	-	-	-	-			-	-	-	-
April	2.06	2.71			3.67	4.84			0.09	0.14	0.11	0.16
May	1.31	1.28	3.11	3.05	2.95	2.89	2.45	2.41	0.12	0.09	0.13	0.11
June	0.80	0.69			2.06	1.66			0.14	0.10	0.16	0.10
July	1.74	2.01			3.32	3.65			0.13	0.12	0.08	0.07
Mean value	1.69	2.02	2.80	3.03	2.57	3.10	2.03	2.30	0.13	0.16	0.14	0.16
(± s.d.)	±1.04	±1.39	±0.41	±0.05	±1.31	±1.89	±0.40	±0.58	±0.09	±0.14	±0.10	±0.17

Table 6. Loss on ignition in % of the dry weight at 550°C. When a $A_{01} + A_{02}$ values was constructed it was made by taking $0.75 \times A_{01}$ value (= top 2.5 cm of the humus) + $0.25 \times A_{02}$ value (= the lowest cm of the humus).

Date	A_{00}	A_{01}	$A_{01} + A_{02}$	A_{02}	A_2	B
May 21 1974		91.2	78.4	39.8	2.7	5.5
June 5		91.			6.2	
June 22	96.3					
Aug 20	95.0	86.2	71.7	28.3	3.6	4.5
Nov 20	97.9	63.5	73.3	28.5	3.5	3.6
Jan 21 1975	95.5		84.1			
Feb 20	94.4	82.7	74.9	51.3	3.1	4.2
April 29	94.9		87.4			
May 27	94.2	88.1	80.9	59.4	2.5	4.0
July 23	95.6		86.9		3.8	4.3
	95.5±1.2 (s.d., n=8)	83.8±10.4 (s.d., n=6)	74.7±6.4 (s.d., n=8)	41.5±13.8 (s.d., n=5)	3.6±1.2 (s.d., n=7)	4.4±0.6 (s.d., n=6)

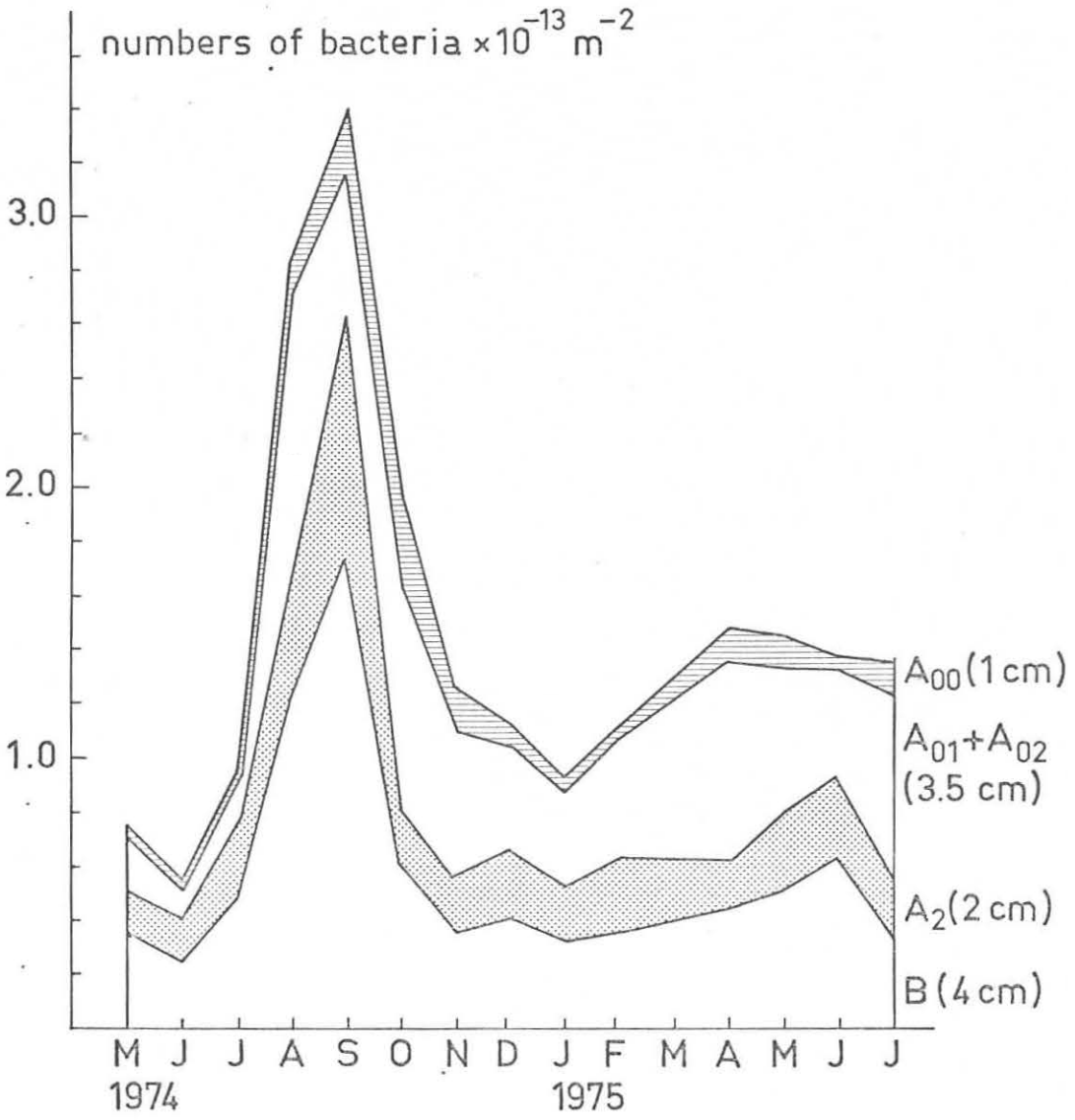

Figure 1. Numbers of bacteria per m^2 and the distribution in the litter (A_{00}), humus ($A_{01}+A_{02}$), bleached mineral layer (A_2) and the enriched mineral layer (B) down to 10.5 cm from the surface at Ih VA. For sampling dates see Table 1.

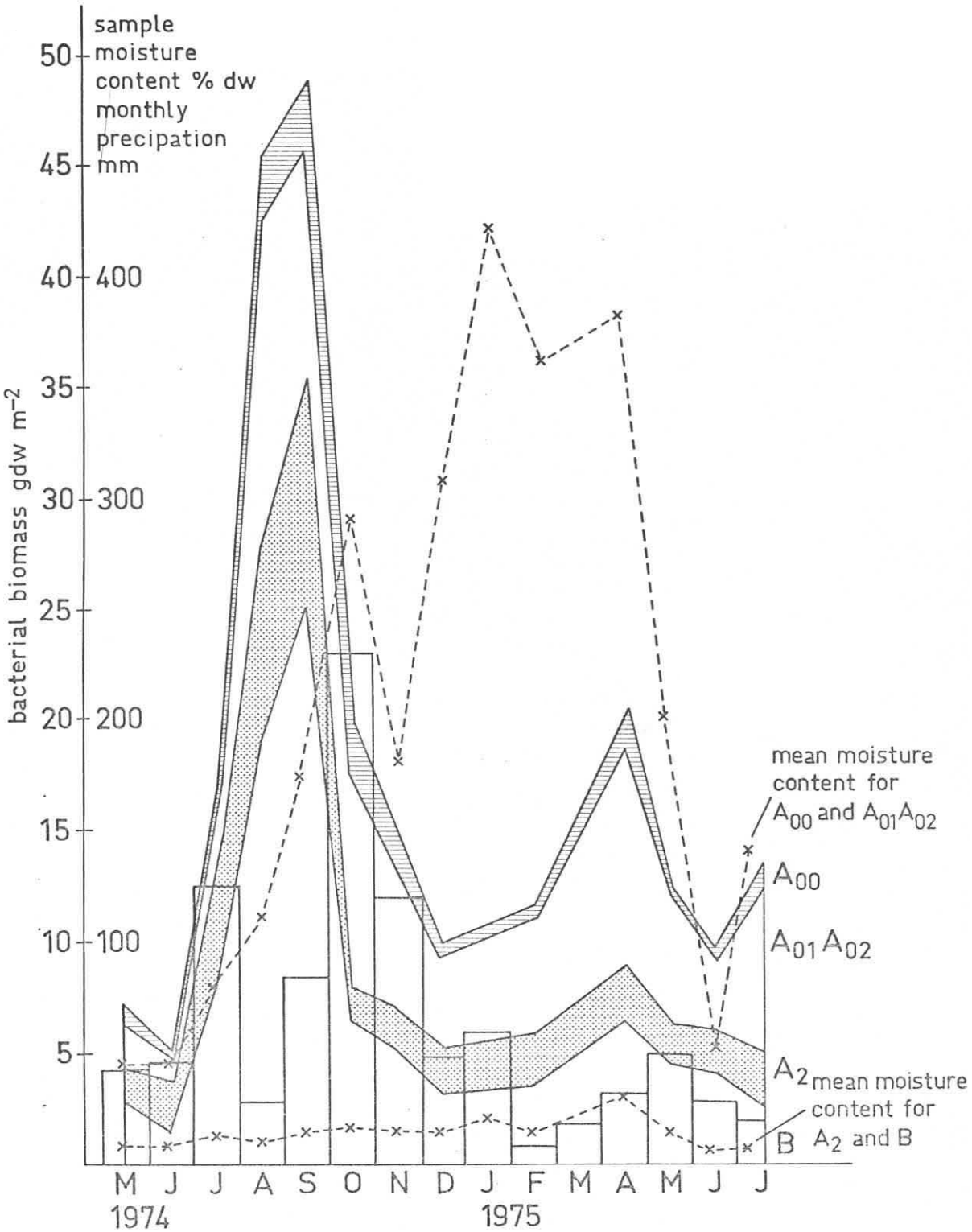

Figure 2. Amount of bacterial biomass per m^2 and its distribution in the litter, humus, bleached and enriched mineral layers down to 10.5 cm from the surface at Ih VA. Monthly precipitation is given as columns. For sampling dates and for individual moisture values for the four layers see Table 1. In the figure, for clarity only the mean of the moisture of mineral and organic layers are plotted as they did not differ much.

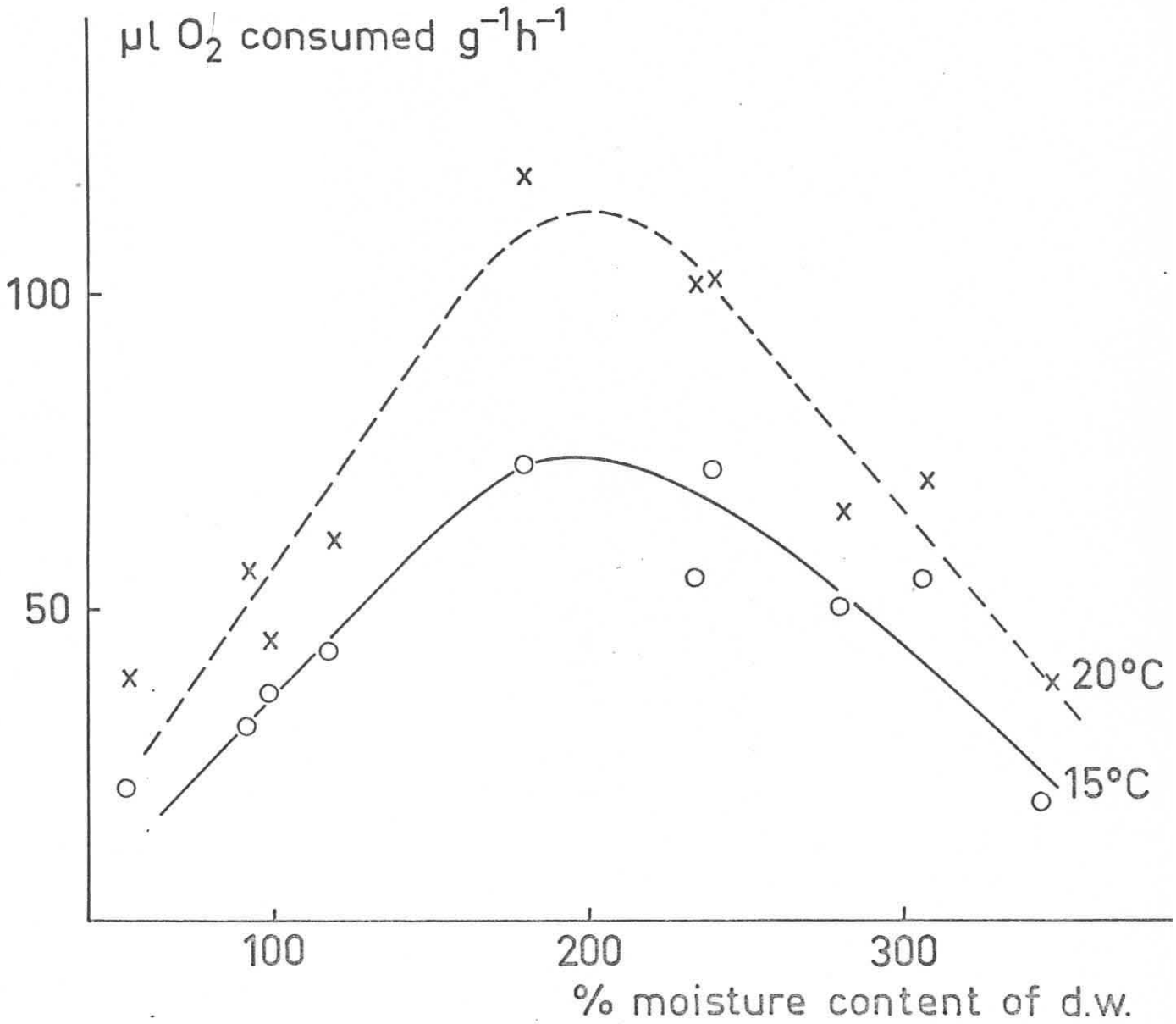

Figure 3. Respiration rate of the humus layer at different adjusted moisture levels measured by Gilson respirometry at 15°C and 20°C.